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WAR DEPARTMENT

PHYSICAL SCIENCES DIVISION

CHEMICAL CORPS BIOLOGICAL LABORATORIES

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Project on Marine Biology DA-18-064-CML-471

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Plankton Studies

Field

During this period no routine sampling was done. The only collection trips were for sea water and tideland mud for the media.

Laboratory

Further studies were made on a better method for aerating the 20-liter bottles.

Method 4 is a direct aeration by bubbling air into the media from an aquarium pump. The tiny bubbles rising to the surface from the glass filter proved too disturbing and numbers of Gonyaulax died.

Method 5 is the same direct method but protecting the volume of the media by a glass sleeve over the aeration tube. This proved more satisfactory. Although this method gives more satisfactory results, the larger bottles still do not equal the test tube cultures.

Having a constant water bath temperature 12° - 16° C and a constant aeration, as Method 5, the next step is the standardization of the media, such as an artificial sea water media.

Extraction methods

With small volumes the routine has been "X" method--centrifuge culture, decant the media, add equal volume of 0.1 N HCl, boil for 10 minutes. All the test tube cultures are tested in this manner.

When larger volumes were used it was necessary to find a rapid method of concentrating the Gonyaulax. The following methods were tried:

"O". Sintered glass plate, filtration--too slow and plate easily clogged

"Y" Celite column, washed with distilled H_2O, culture filtered by vacuum. The Celite and Gonyaulax extracted by boiling 10 minutes with 50% Ethanol + 1 m/liter of conc HCl.

Two other methods have been tried in the attempt to find a better extraction method.

When the culture was put through air-driven Sharples at 50,000 rpm at the rate of 100-200 ml/minute, the procedure was very easy but the loss in poison when scraping the Sharples bowl was great.

The extraction of large volumes was best effected by vacuum filtration through a large surface, small volume of Celite No. 512 filter. The poison is then eluted without vacuum with 50% acidified ethanol as a part of the first orange-colored fraction. For example, Experiment No 2: the Gonyaulax were filtered out by the preceding method. The poison is then eluted with 50% acid ethanol in the orange-colored fraction.

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Eluate	No. 1	orange pigment	2.8 ml	25 Mu/ml
	No. 2	yellow	100 ml	<10 Mu/ml
	No. 3	green	100 ml	<10 Mu/ml
	No. 4	green	100 ml	<10 Mu/ml

The majority of the poison comes out in the first eluate, but in order to be sure if this is the best method it is to be run in parallel with the direct method X

The test tube cultures in the small tank showed a high of 12,200 dinoflagellates per ml in media No. 6, while the best poison of 0.55 ml/Mu was produced by 3,370 Gonyaulax for a mouse unit. In February the bottom of the small tank rusted out and was sent out for repairs. Small flasks were used in the large tank.

During the period the best growth in the large bottles was with media No. 6 - 7,900 Gonyaulax per ml. The best poison was 1.8 ml/Mu or 5,807 Gonyaulax to make a mouse unit.

K. T. Meglen